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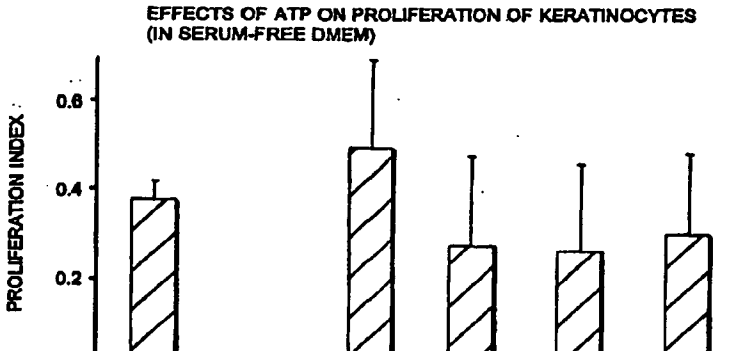

(57) Abstract

The invention relates to P2 agonists and antagonists or a compound which will stimulate or inhibit endogenous adenosine triphosphate (ATP) production, and more particularly to novel medical uses for same. More particularly still it relates to treating skin conditions characterised by hyperproliferation of keratinocytes, including for example, keloid formation, dermatitis and psoriasis or enhancing wound healing. The invention provides the use of an agonist or antagonist of a type P2–receptor or a compound which will stimulate or inhibit adenosine triphosphate (ATP) production for the manufacture of a medicament for treating wounds or skin conditions characterised by hyperproliferation of keratinocytes or acanthosis. It also provides a pharmaceutical composition comprising a growth factor, a pharmaceutically acceptable carrier and either an agonist of a P2Y receptor or a compound which will stimulate adenosine triphosphate (ATP) production.

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DESCRIPTION

P2 RECEPTOR AGONISTS, ANTAGONISTS AND MODULATORS OF ENDOGENOUS ATP RELEASE

The present invention relates to P2 receptor agonists, antagonists and modulators of endogenous ATP release and more particularly to novel medical uses for same.

It has been suggested that P2 receptor agonists may be useful antineoplastic agents. US 5415873 contemplates formulating pharmaceutical compositions comprising an effective anti cancer amount of a P2 receptor agonist and a pharmaceutically acceptable carrier.

The present invention is in contrast concerned with treating skin conditions characterised by hyperproliferation of keratinocytes, including for example, keloid formation, dermatitis and psoriasis, or enhancing wound healing.

Currently, there are no completely effective topical treatments that combat keloid formation, dermatitis, psoriasis and related skin disorders although glucocorticoids, vitamin D metabolites and analogues, retinoids and coal tar preparations are partially effective in some situations. However, these therapies are not without detrimental side effects.

Similarly whilst topical applications of growth factors, for example, epidermal growth factor (EGF),

Transforming Growth Factor Beta (TGFβ) and parathyroid hormone-related protein (PTHrP) are under trial as aids to wound healing, they are expensive to produce, and because of their size are not easily absorbed into the deeper layers of the epidermis.

It is an aim of the present invention to identify compounds which may be useful in treating skin conditions characterised by hyperproliferation of keratinocytes or which enhance wound healing.

According to the present invention there is provided the use of an agonist or antagonist of a type P2- receptor or a compound which will stimulate or inhibit endogenous adenosine triphosphate (ATP) release for the manufacture of a medicament for treating a wound or a skin condition characterised by hyperproliferation of keratinocytes or acanthosis.

Preferably the agonists or antagonists or modulators of endogenous ATP release will be incorporated into a pharmaceutical composition for topical administration. The composition may take the form of a solution, emulsion, suspension, ointment, cream or aerosol.

It is believed that the P2 agonists and antagonists or modulators of endogenous ATP release influence the proliferation of epidermal cells such that they may be of benefit in treating some skin disorders, including for example, keloid formation,

dermatitis and psoriasis which are characterised by hyperproliferation of keratinocytes or acanthosis. In these conditions it would be desirable to inhibit proliferation and promote differentiation of keratinocytes. In contrast, acceleration of keratinocyte proliferation would be required to enhance wound healing.

The invention is based on the applicants discovery that there is an abundance of P2Y receptors including for example P2Y1, P2Y2, P2Y4 and P2Y6 receptors in the proliferative cells of the epidermis and that ATP is released from keratinocytes into the extracellular environment in physiological and pathological conditions.

By selecting those compounds which act as either agonists or antagonists of P2Y receptors expressed by keratinocytes of skin or those compounds which modulate endogenous ATP release it is possible to either inhibit or stimulate keratinocyte proliferation, directly and/or indirectly regulating proliferation of this cell type, by modulating cellular differentiation.

Representative agonists for these receptor subtypes include both adenosine and uridine containing compounds such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine triphosphate (UTP), uridine diphosphate (UDP) and derivatives thereof.

Antagonists of these receptors include suramin, reactive blue and pyridoxal phosphate and derivatives including, pyridoxal phosphate 6 azophenyl 2',4' disulphonic (PPADS) and iso-PPADS.

A preferred modulator of endogenous ATP release is the inhibitor glibenclamide.

Using established methodology, *in situ* localisation of P2Y2 receptor transcripts in sections of skin revealed an intense localisation over the actively proliferating keratinocyte layer, the stratum basale. Progression through the more differentiated layers of the epidermis resulted in a sharp decrease in P2Y2 receptor expression. In addition, the inventors have demonstrated by reverse transcription-linked polymerase chain reaction, the expression of multiple P2Y receptor subtypes, the P2Y1, P2Y2, P2Y4, P2Y6, by primary human keratinocytes *in vitro* culture. The functional viability of these expressed receptors was confirmed by loading primary keratinocytes with fura-2 am and recording elevations of intracellular calcium in response to appropriate nucleotide agonists, an event associated with terminal keratinocyte differentiation. In addition it has been demonstrated in other cell types that P2 receptor subtype expression is a differentiation dependent process. Hence, receptor specific agonists/antagonists could be used to control keratinocytes at different states of differentiation,

and/or influence their progression.

Keratinocytes cultured in vehicle only, showed high basal levels of proliferation, as assessed in a routine in vitro assay utilising incorporation of the thymidine analogue bromo deoxyuridine, consistent with the presence of actively proliferating de-differentiated keratinocytes. ATP or UTP treatment of cells in the absence of serum reduced the proliferative response. In contrast ATP or UTP (1μM or 100μM) and 10% FCS co-stimulation significantly stimulated proliferation of cultured human keratinocytes - see Figs. 1 and 2. The non-hydrolysable analogue of ATP, ATPγS, produced a similar pattern of keratinocyte proliferation in the presence or absence of serum.

The inventors have demonstrated that primary human keratinocytes release physiological concentrations of ATP using a firefly luciferase/luciferin detection system capable of assessing ATP release from bone cells in real time. The detection system comprised of a circular stainless steel chamber (diameter 14mm and depth 0.65 mm) capable of receiving a 13 mm diameter glass cover slip. The chamber was maintained at 37°C by circulating water and housed in a refrigerator, cooled to 2-4°C, containing a photomultiplier for real time luminescence detection. A perfusion system allowed the chamber to be filled and to generate fluid flow-induced shear forces. Luciferin and luciferase

were both diluted in Hepes buffered medium at concentrations of 10μM and 1μg/ml respectively. The system was positioned in a dark room to give a low background signal. Using this system the inventors have demonstrated that glibenclamide, inhibits constitutive ATP release from human keratinocytes, implicating ATP binding cassette proteins or associated proteins or ion channels in mediating the release of ATP from keratinocytes. In addition the inventors demonstrate that shear rates as low as (30s-1) enhances ATP release from cells.

Topical application of these agonists, antagonists or modulators will provide a means of treating skin disorders characterised by hyperproliferation of keratinocytes, e.g. keloid formation, dermatitis or psoriasis. Furthermore, these compounds may be used to accelerate the process of wound healing by stimulating the proliferation of keratinocytes.

Alternatively, topical application of compounds that regulate the release of ATP from keratinocytes may have a beneficial effect in disorders associated with ATP-stimulated keratinocyte hyperproliferation, or as a means of increasing keratinocyte proliferation.

The applicants have also determined that P2Y receptor agonists have a beneficial effect when used in combination with growth factors, such as, for example TGFβ, EGF and PTHrP.

According to a further aspect of the present invention there is provided a pharmaceutical composition comprising a growth factor, a pharmaceutically acceptable carrier and an agonist of a P2Y receptor or a compound which will stimulate endogenous adenosine triphosphate (ATP) release.

The composition is adapted for topical application to the skin.

The applicants have demonstrated that ATP and other extracellular nucleotides potentiate the activity of growth factors in vitro.

Thus, for example, expression of the c-fos gene was studied in human cell line SaOS-2. Expression of the gene is associated with cellular proliferation and differentiation. Maximum induction (100%) was taken to be that achieved with a 10% solution of foetal calf serum (FCS) and zero induction (0%) that achieved in a α modified eagles medium (serum free). By extracting RNA from the cells and using Northern analysis the % induction could be determined using a densitometer. The results are approximated in Table 1 below and shown in more detail in Fig. 3 which depicts the Northern analysis of total RNA extracted from SaOS-2 cells following treatment with agents 1 to 7 described in Table 1. Each value for c-fos induction is represented as a percentage of the positive control; Total RNA extracted from SaOS-2 cells grown in a α modified

Eagles Medium (α-MEM) supplemented with 10% foetal calf serum (FCS).

Table 1

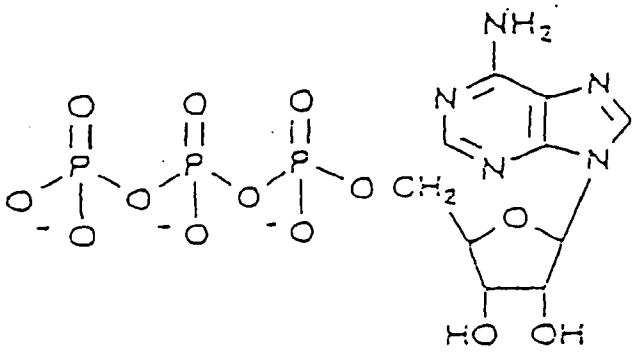
	TREATMENT	C-FOS INDUCTION AS % OF +VE CONTROL
1	10% Foetal calf serum (+ve control)	100
2	PTH (100 ng/ml)	30
3	α-MEM (+ ve control)	0
4	ADP + PTH	80
5	ADP (10 μM)	10
6	ATP + PTH	60
7	ATP (10 μM)	5

The compounds of this invention are preparations which contain specific agonists or antagonists of P2Y receptors or modulators of endogenous ATP release for topical application to skin. Some of these agonists are exemplified below and include the purine nucleotides e.g. ATP, and the pyrimidine nucleotides UTP and UDP and derivatives of each of the above. Antagonists include suramin, reactive blue and pyridoxal phosphate and derivatives including, pyridoxal phosphate 6 azophenyl 2',4' disulphonic

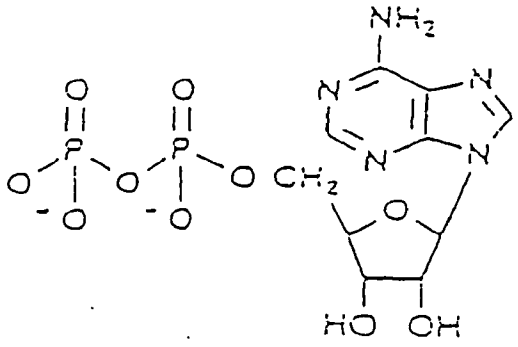
(PPADS) and iso-PPADS. Modulators of endogenous ATP release include the ATP inhibitor glibenclamide. These compounds are to be used specifically for the treatment of skin disorders characterised by hyperproliferation of keratinocytes or as an aid to the wound healing process.

The purine nucleotides include:

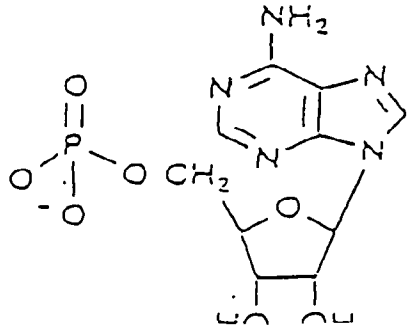
1. ATP



2. ADP

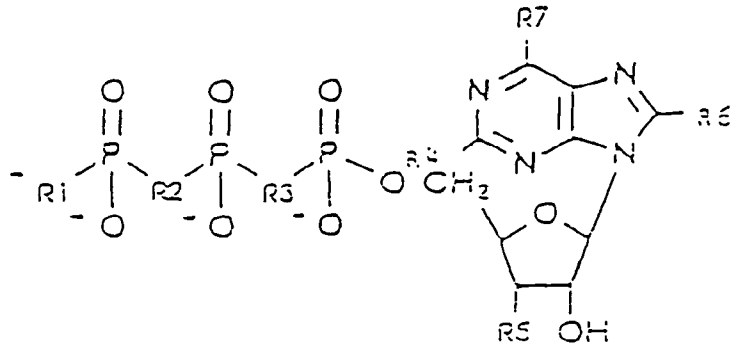


3. AMP



and derivatives thereof.

Known derivatives include compounds of the general formula I.

I 

Where

R1 is S or O,

R2 is CH_2 or O,

R3 is CH_2 or O, and the substituents R4 to R7 are, if present:

R4 is CH_3S,

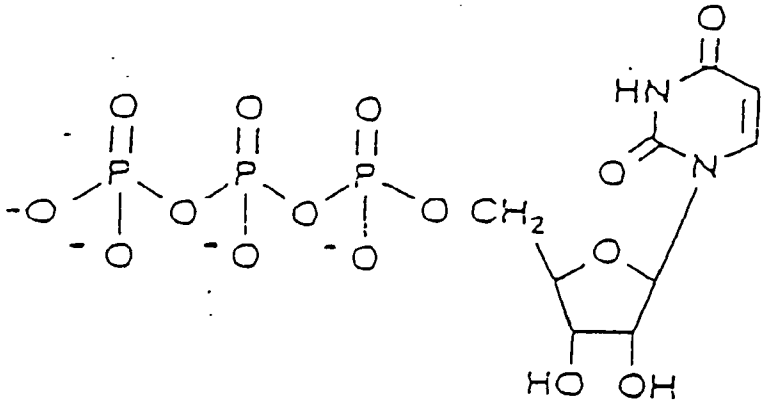
R5 is CH_3NONH,

R6 is $NH(CH_2)_6NH_2$, and

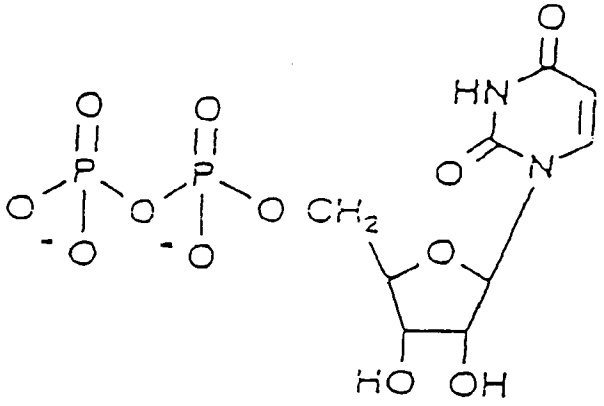
R7 is NH_2 or $NHCH_3$.

The pyrimidine nucleotides include:

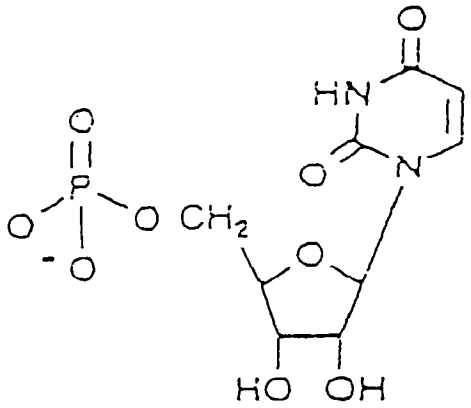
1. UTP



2. UDP



3. UMP



Known derivatives include compounds in which the terminal oxygen atom of the phosphate group is replaced with a sulphur atom.

The compounds of the present invention can be used for the treatment of human or animal conditions. It is envisaged that the active compounds will be formulated into a pharmacological composition comprising an effective proliferative/antiproliferative agent and a pharmacologically acceptable carrier. The effective amount of compound will vary between 1pM and 1 mM depending upon the subject and the condition of this subject and the particular compound. Pharmaceutically acceptable carriers are materials useful for the purpose of administering the medicament, which are preferably sterile and non-toxic and may be liquid or gaseous, which are otherwise inert and medically acceptable, and are compatible with the active ingredients. The pharmaceutical compositions may further comprise other ingredients, such as, preservatives.

These pharmaceutical compositions may be administered topically in the form of a solution, emulsion, suspension, ointment, cream or aerosol. The composition may contain the compound in any proportion by weight of the total composition.

CLAIMS

1. The use of an agonist or antagonist of a type P2- receptor or a compound which will stimulate or inhibit endogenous adenosine triphosphate (ATP) release for the manufacture of a medicament for treating a wound or a skin condition characterised by hyperproliferation of keratinocytes or acanthosis.

2. The use of an agonist of a type P2- receptor or a compound which will stimulate adenosine triphosphate (ATP) production as claimed in claim 1 for the manufacture of a medicament for enhancing wound healing.

3. The use of an antagonist of a type P2-receptor or a compound which will inhibit adenosine triphosphate (ATP) production as claimed in claim 1 for the manufacture of a medicament for treating a skin condition characterised by hyperproliferation of keratinocytes or acanthosis.

4. The use of an antagonist of a type P2-receptor or a compound which will inhibit adenosine triphosphate (ATP) production as claimed in claim 3 for the manufacture of a medicament wherein the skin condition is characterised by keloid formation.

5. The use of an antagonist of a type P2-receptor or a compound which will inhibit adenosine triphosphate (ATP) production as claimed in claim 3 for the manufacture of a medicament wherein the skin

condition is dermatitis.

6. The use of an antagonist of a type P2-receptor or a compound which will inhibit adenosine triphosphate (ATP) production as claimed in claim 3 for the manufacture of a medicament wherein the skin condition is psoriasis.

7. The use of an agonist or antagonist of a type P2- receptor or a compound which will stimulate or inhibit adenosine triphosphate (ATP) production as claimed in any of the preceding claims for the manufacture of a medicament wherein the medicament is for topical administration.

8. The use of an agonist or antagonist of a type P2- receptor or a compound which will stimulate or inhibit adenosine triphosphate (ATP) production as claimed in claim 7 for the manufacture of a medicament wherein the medicament is a solution, emulsion, suspension, ointment, cream or aerosol.

9. The use of an agonist of a type P2- receptor as claimed in claim 2 for the manufacture of a medicament wherein the agonist is an extracellular nucleotide.

10. The use of an agonist of a type P2- receptor as claimed in claim 9 for the manufacture of a medicament wherein the extracellular nucleotide is an adenosine or uridine containing compound.

11. The use of an agonist of a type P2- receptor

as claimed in claim 10 for the manufacture of a medicament wherein the adenosine containing compound is selected from adenosine triphosphate (ATP), adenosine disphosphate (ADP), and adenosine mono phosphate (AMP), and derivatives thereof and the uridine containing compound is selected from uridine triphosphate (UTP), uridine diphosphate (UDP), and uridine mono phosphate (UMP) and derivatives thereof.

12. The use of an antagonist of a type P2-receptor as claimed in claim 3 for the manufacture of a medicament wherein the antagonist is selected from the group consisting of suramin, reactive blue, pyridoxal phosphate and derivatives thereof.

13. The use of an antagonist of a type P2-receptor as claimed in claim 12 for the manufacture of a medicament wherein the derivatives are selected from the group consisting of pyridoxal phosphate 6 azophenyl 2', 4' disulphonic (PPADS) and iso-PPADS.

14. The use of an agonist of a type P2- receptor or a compound which will stimulate adenosine triphosphate (ATP) production as claimed in claim 2 or any of claims 7 to 11 for the manufacture of a medicament which further comprises a growth factor.

15. The use of an agonist of a type P2- receptor or a compound which will stimulate adenosine triphosphate (ATP) production as claimed in claim 14 for the manufacture of a medicament wherein the growth

factor is selected from the group consisting of TGFβ, EGF and PTHrP.

16. The use of an agonist or antagonist of a type P2 receptor or a compound which will stimulate or inhibit adenosine triphosphate (ATP) production as claimed in any of the preceding claims wherein the agonist or antagonist or a compound which will stimulate or inhibit endogenous adenosine triphosphate (ATP) release is present in an amount from 1pM to 1 mM.

17. A pharmaceutical composition comprising a growth factor, a pharmaceutically acceptable carrier and an agonist of a P2Y receptor or a compound which will stimulate endogenous adenosine triphosphate (ATP) release.

18. The pharmaceutical composition as claimed in claim 17 wherein the agonist of the P2Y receptor or a compound which will stimulate adenosine triphosphate (ATP) production is present in an amount of from 1pM to 1mM.

19. The use of glibenclamide for the manufacture of a medicament for treating a skin condition characterised by hyperproliferation of keratinocytes or acanthosis.

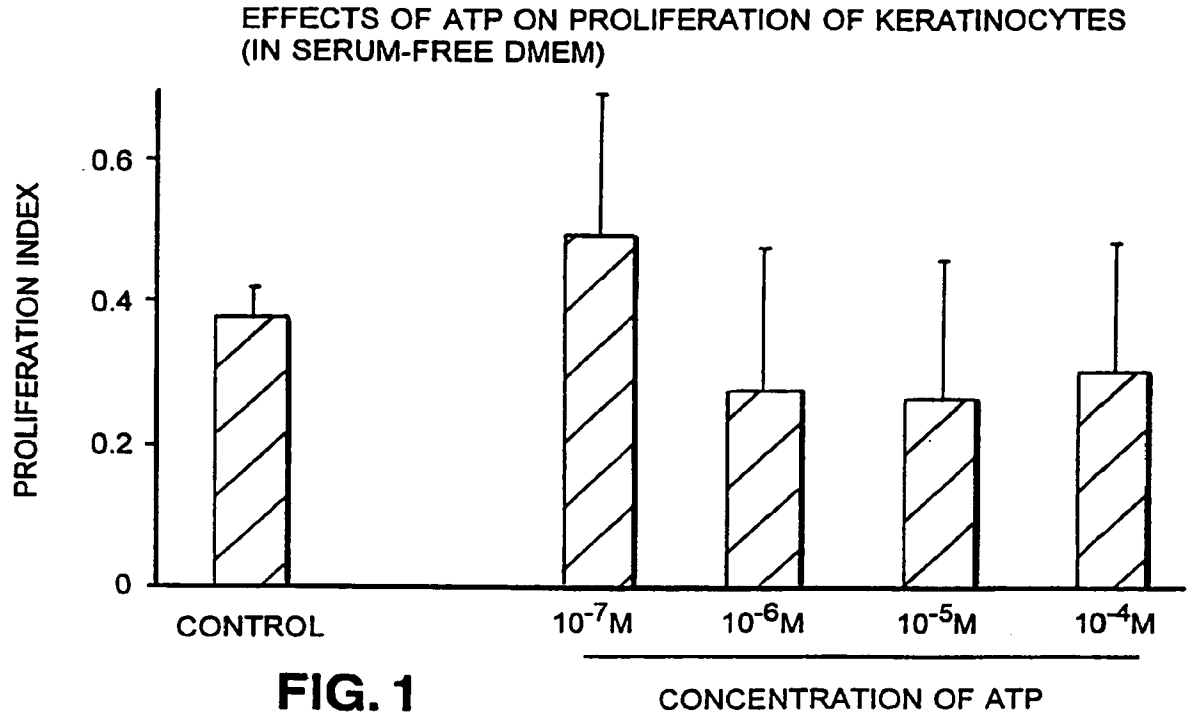

EFFECTS OF ATP ON PROLIFERATION OF KERATINOCYTES
(IN SERUM-FREE DMEM)
PROLIFERATION INDEX
0.6
0.4
0.2
0
CONTROL
10-7M
10-6M
10-5M
10-4M
CONCENTRATION OF ATP

FIG. 1

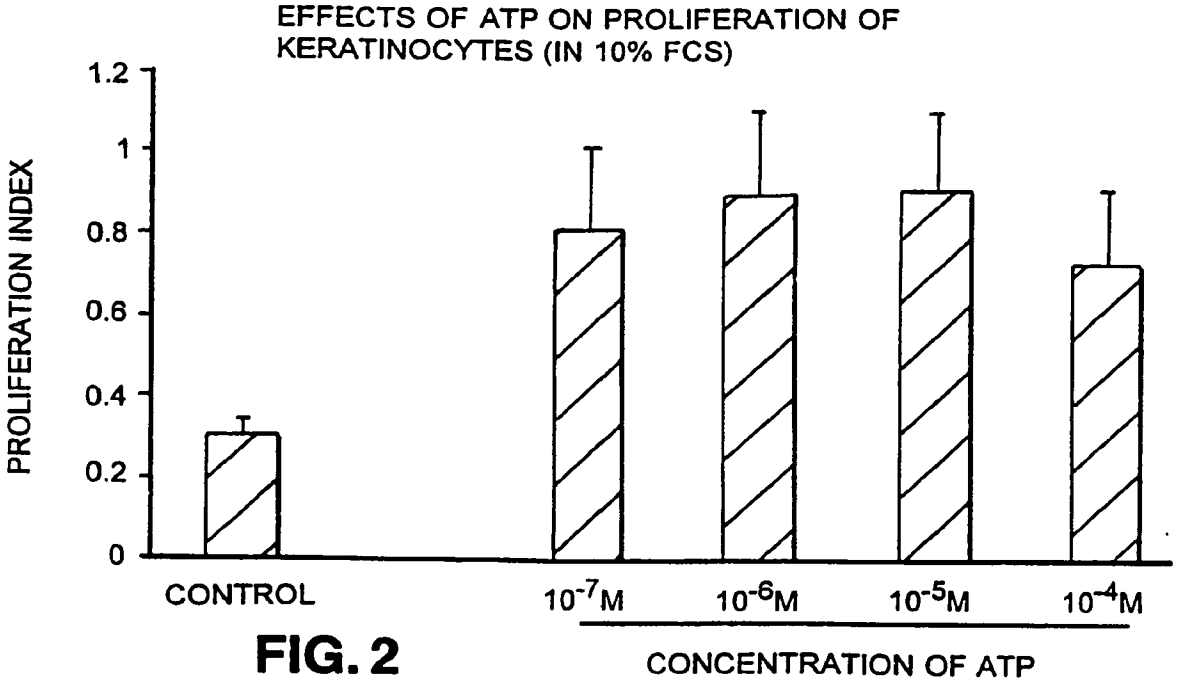

EFFECTS OF ATP ON PROLIFERATION OF
KERATINOCYTES (IN 10% FCS)
PROLIFERATION INDEX
1.2
1
0.8
0.6
0.4
0.2
0
CONTROL
10-7M
10-6M
10-5M
10-4M
CONCENTRATION OF ATP

FIG. 2

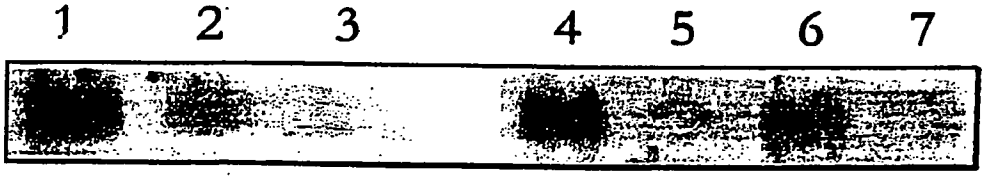


FIG. 3